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Assimilating transcriptional profiles from CCLE lesional skin and peripheral blood offers a comprehensive model of disease pathogenesis

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The importance of skin manifestations in lupus erythematosus (LE) makes it vital to investigate cutaneous disease based on molecular criteria. To this end, our strategy includes microarray-based gene expression analyses of lesional skin and blood of chronic cutaneous lupus erythematosus (CCLE) patients to illuminate disease mechanisms and clarify the molecular genetic basis of disease heterogeneity. Here, we integrate gene expression data from both environments and use a functional and genetic approach to better understand pathomechanisms of disease. There is a significant upregulation of apoptosis and interferon response in both skin and blood of CCLE patients consistent with reported findings in SLE supporting the hypothesis that these key pathways are relevant across the broad spectrum of lupus. Taken together, our data suggests a comprehensive model of systemic and local disturbances orchestrated as three distinct phases in the autoimmune response: 1) initiation, 2) amplification of the immune response, and 3) target damage. Of interest, we observe an enrichment for heightened immune surveillance/initiation related processes in lesional skin than in peripheral blood of CCLE patients. Further, we identify seven regions of overlap between transcriptional "hot spots" found in CCLE blood and lesional skin, with several genes that do not correspond with reported SLE dysregulated genes or putative risk loci, thus potentially harboring susceptibility loci distinct for CCLE.

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DDX6 orchestrates human epidermal progenitor function through the mRNA degradation and translation pathways.

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Although diseases that involve alterations in epidermal growth and differentiation impact every one in five people, the basis for how epidermal progenitor cells suppress premature differentiation and promote self-renewal is unclear. Here, we show that DDX6 is necessary to maintain self-renewal of the progenitor cells that reside in the basal layer of the epidermis. Depletion of DDX6 resulted in loss of progenitor cell function due to premature differentiation and loss of proliferation. In-vivo progenitor cell competition assays demonstrated that DDX6 mediates self-renewal through cell intrinsic mechanisms. Gene expression profiling revealed that DDX6 is necessary to sustain proliferation while actively suppressing differentiation. To promote proliferation and self-renewal, DDX6 facilitates the translation of regulators of proliferation (*CDK1* and *HMGB2*) and epigenetic factors necessary for self-renewal such as *EZH2* and *ACTL6a*. DDX6 through association with mRNA binding protein, YBX1, bind to stem loops found in the 3'UTRs of these mRNAs and recruits them to EIF4E to promote translation and thus maintains the proliferative and self-renewal capacity of progenitor cells. To actively suppress differentiation, DDX6 regulates a GC rich region in the 5'UTR of *KLF4*, a transcription factor necessary for epidermal differentiation, and promotes its degradation through mediators of mRNA degradation such as EDC3. Collectively, our results suggest that DDX6 complexes maintain progenitor cell fate by facilitating the translation of mRNAs involved in proliferation and self-renewal while also targeting differentiation inducing mRNAs for degradation.

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Trans-ethnic genome-wide meta-analysis identifies multiple novel associations and reveals ethnic heterogeneity of psoriasis susceptibility

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In order to extensively identify the susceptibility genes and characterize the ethnic heterogeneity of psoriasis, we conducted the largest trans-ethnic genome-wide meta-analysis (GWMA) of psoriasis in 15,369 cases and 19,517 controls of Caucasian and Chinese ancestries. We identified four novel associations at rs9533962 (OR=1.12, $P=7.53\times10^{-11}$, *LOC144817*), rs34394770 (OR=1.16, $P=2.65\times10^{-8}$, *COG6*), rs8128234 (OR=1.17, $P=3.74\times10^{-8}$, *RUNX1*), and rs28512356 (OR=1.17, $P=4.31\times10^{-8}$, *TP63*), as well as three novel secondary associations within *IFIH1* (rs3747517, $OR_{condition}=0.78$, $P_{condition}=1.52\times10^{-18}$) and *IL12B* (rs2853694, OR=1.23, $P=4.24\times10^{-10}$; rs4921493, OR=1.23, $P=3.52\times10^{-13}$). Fine-mapping analysis of MHC region had demonstrated an important role for all three *HLA* class I genes and a complex and heterogeneous pattern of *HLA* associations between Caucasian and Chinese populations. Further trans-ethnic comparison suggested population-specific or allelic heterogeneity for eleven loci, and these population-specific effects contribute significantly to the ethnic diversity of psoriasis prevalence. This study not only provides novel biological insights into the involvement of immune and keratinocyte development mechanism, but also demonstrates a complex and heterogeneous genetic architecture of psoriasis susceptibility across ethnic populations.

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RNA recognition motif of LEMD3 as a key player in the pathogenesis of Buschke–Ollendorff syndrome

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Buschke–Ollendorff syndrome (BOS) is an autosomal dominant disease characterized by disseminated connective tissue nevi of elastic type and osteopoikilosis. BOS results from loss-of-function mutations in the *LEMD3* gene, which encodes a potent negative regulator of the transforming growth factor-β and bone morphogenic protein signaling pathways. Although the pathogenic role of *LEMD3* mutations in BOS is not fully understood, the RNA recognition motif (RRM) of LEMD3, which binds to R-Smads, has been shown to be critical in the function of the protein *in vitro*. However, the importance of the RRM in the pathogenesis of BOS has not been verified clinically. Here we report a two-generation Japanese family with BOS. The proband and his younger brother had asymptomatic plaques on the trunk, which were histopathologically confirmed to be connective tissue nevi. Pelvis and hip radiographs revealed "spotted bones", which is consistent with osteopoikilosis; their mother also had osteopoikilosis, without any cutaneous lesions. We performed a mutation analysis of *LEMD3* using genomic DNA extracted from peripheral blood leukocytes. Direct sequencing of all exons and exon/intron boundaries of *LEMD3* identified the novel frameshift mutation *c.2495delG*, which causes a premature termination codon (PTC) at 31 amino acids downstream of the mutation (*p.Gly832Valfs*31*) in the RRM. All the affected family members are heterozygous for the mutation. Since the PTC occurs in the last exon of the gene, the mutant *LEMD3* mRNA is predicted to escape nonsense-mediated mRNA decay (NMD). Indeed, qRT-PCR analysis demonstrated no decrease in the amount of *LEMD3* mRNA in the affected mother. In addition, qRT-PCR analyses for LEMD3-associated genes revealed the reduced expression of *Smad6* mRNA, whereas the expression level of *Smad7* mRNA was not diminished. Thus, this study is the first to suggest that loss of the RRM, and not necessarily loss of the whole LEMD3 protein by NMD, could cause characteristic BOS symptoms through imbalance of Smad6 and Smad7 expression.

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Epigenetic modulation of skin equivalents by cosmetic ingredients

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Numerous topical ingredients are available for formulating cosmetic products. A variety of biological assessment methods aids in the selection of cosmetic ingredients for finished products. Differences in the source or manufacturing process used to obtain the ingredient and the blending of ingredients impact biological efficacy. Modulation of gene expressions is commonly used to evaluate biological activities. Epigenetic changes play a significant role in how genes are expressed and translated into functional proteins. Epigenetics represents the heritable changes without altering DNA sequences; it is critical in biological functions, since epigenetics allows tissue-specific changes in gene expression. Examples of epigenetic modifications are DNA methylation, histone modification and regulatory RNAs. Cosmetic ingredients are evaluated for epigenomics, utilizing the DNA methylation arrays for more global assessments or individual genes/targets by looking at the specific promoter regions for histone modification or methylation patterns. High throughput screening methods can also be used to measure expression of microRNAs, small non-coding regulatory RNAs that play a significant role in gene expression. A qPCR-based screening method was used to examine microRNAs with established roles in skin biology. This method was used to investigate how combinations of topical ingredients modulate gene and microRNA expression in full-thickness skin equivalent models. Results showed changes in several mRNAs gene expression, with some correlated changes in microRNAs, such as SIRT1 stimulation through the inhibition of miR-9. Additional correlations between epigenetic modulation and mRNA gene expression that influence skin aging are being investigated.

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The lncRNA FLJ46906 alters expression of aging-associated proteins through binding to AP-1 and NF-κB

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Aged and young cells are characterized by different gene expression profiles, but the mechanisms of altered gene expression in aged cells remain incompletely understood. Long non-coding RNA (lncRNA) is a recently described class of RNA. Some lncRNAs are involved in gene regulation, e.g. during development and tumorigenesis, but the functions of most of the lncRNAs remain unknown. We hypothesized that lncRNAs are responsible for altered gene expression in aged cells. Using dermal fibroblasts from a young and an old donor and a DNA microarray that contains probes for more than 7,000 lncRNAs, we screened for differentially expressed lncRNAs. Out of 63 either up- or down-regulated lncRNAs, one lncRNA, called FLJ46906, was confirmed by qPCR to be consistently upregulated (3-fold) when comparing cells from several young and old donors. The function of this lncRNA was unknown. Some lncRNAs affect expression of neighboring genes, but the expression of protein-coding genes in the vicinity of FLJ46906 was not found to be altered. To discover its function, FLJ46906 was then knocked down in fibroblasts using siRNA to investigate its impact on aging-associated proteins. While the expression of MMPs and COL1α were not affected, expression of IL-1, 6, 8, TNF-α and p21 mRNAs was decreased and expression of TGF-β and elastin mRNAs increased. As the expression of these genes is typically altered in aged cells, these results suggest that FLJ46906 mediates aging in fibroblasts. As these proteins have in common that they are regulated by the inflammatory transcription factors AP-1 and NF-κB, we hypothesized that FLJ46906 binds to these factors. This was confirmed by results of RNA immunoprecipitation assays that showed strong and specific binding of FLJ46906 to AP-1 and NF-κB. In summary, our data demonstrate that the lncRNA FLJ46906 alters expression of crucial, aging-associated proteins through binding to AP-1 and NF-κB and support the hypothesized role of lncRNAs in the aging process.

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TALEN-induced mutations confirm Col17a1 as a genetic modifier of junctional epidermolysis bullosa in mice

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Normal allelic variation in the NC4 domain encoded by Col17a1 exon 50 affects the onset and severity of Lamc2^{jeb} induced junctional epidermolysis bullosa (JEB) in mice. Mouse mutations resulting from TALEN targeting of Col17a1 intron 50-51 and exon 50, including a 2 SNP replacement event confirming the modifier role of S1275G and N1277S AA variation, a 10 bp exonic frame shift deletion which acts as a loss of function mutation, and multiple nested short and long in frame deletions focused on putative key binding sites. Despite the apparent importance of NC4 to collagen 17/laminin 332 binding and the fact that many Col17a1 mutations can result in JEB, mice homozygous for any of these in frame deletions are normal. However, greatly accelerated disease is seen in mice homozygous for the Lamc2^{jeb} mutation when combined with Col17a1^{em1Dcr} whole exon 50 deletions in either the heterozygous or homozygous state. Likewise, heterozygosity for the em8 frame shift mutation alone has no notable effect but combining it with homozygosity for the Lamc2^{jeb} mutation accelerates disease. Combinations of smaller 9-18 bp Col17a1 exon 50 deletions at putative key binding sites with Lamc2$^{jeb/jeb}$ fail to accelerate disease and in some cases actually alleviates symptoms. This expands knowledge of the epistatic interplay between epidermolysis bullosa disease-causing alleles such as Lamc2^{jeb} and relatively innocuous allelic variants of Col17a1 which, when combined, greatly alter disease progression. It also highlights the surprising finding that removal of this relatively important and interactive region of the collagen 17 protein has little or no visible effect in the context of healthy alleles of Lamc2 and other basement membrane components.

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Somatic activating *RAS* mutations cause vascular tumors including pyogenic granuloma

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Lobular capillary hemangiomas (LCH) are vascular tumors featuring lobes of small caliber vessels. While the term LCH is commonly used as a synonym for pyogenic granuloma (PG), LCH as a class encompasses a variety of other lobular vascular lesions including infantile hemangiomas and tufted angiomas. For sporadic lesions such as PG, it has been proposed that lesions may be reactive to trauma, infection, medication and inflammation, though many PGs in uninjured skin have also been described. Treatment is limited to steroids or surgery, and, most recently, β-blockers, although nearly half of vascular tumors including LCH fail to respond. The genetic cause of many LCH lesion types is unknown. Therefore, we employed whole exome sequencing of two vascular tumors with lobular architecture and found somatic activating mutations in *KRAS or NRAS* in each. Recognizing that sporadic PGs share the lobular architecture found in these tumors, we screened 40 archival samples and identified activating *HRAS* mutations in 10% of them (4/40). Our findings of somatic activating *RAS* mutations in these lobular hemangiomas and PG lesions suggest that a subset of vascular tumors are driven by oncogenic *RAS* activation, and provides potential opportunities to develop targeted therapies to current drug-resistant lesions.

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Gasdermin A3 targets mitochondria to mediate keratinocyte necrosis and skin inflammation

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The epidermis forms a critical physical and immune barrier, which could be compromised by deregulated responses of keratinocyte to external stimuli and ensuing inappropriate cell death. *GASDERMIN A* (*GSDMA*) was involved in gastric epithelial apoptosis and has been implicated in airway hyperresponsiveness. Its function in the skin was indicated by dominant mutations in *gasdermin A3* (*Gsdma3*), which caused inflammation-mediated epidermal hyperplasia and hair loss in mice. The mechanisms of Gsdma3's action and pathogenesis are unknown. Here, we showed that Gsdma3 is regulated by intramolecular fold-back inhibition, which is disrupted by dominant mutations in the C-terminal domain. The unmasked N-terminal domain of Gsdma3 then associates with Hsp90 and is delivered to mitochondrial via the mitochondrial import receptor Tom70, where it interacts with the mitohcondrial chaperone Trap1 and causes oxidative stress-mediated mitochondrial permeability transition and caspase-independent cell death. Using an inducible transgenic mouse model, we demonstrated that epidermis-specific expression of Gsdma3 caused spontaneous keratinocyte necrosis and sterile skin inflammation *in vivo*. In primary keratinocytes isolated from the transgenic mice, Gsdma3 expression caused mitochondrial oxidative stress and necrosis. We hypothesized that Gsdma3 functions as a stress sensor, which regulates the susceptibility of keratinocytes to necrosis and its deregulation leads to chronic inflammatory skin diseases.

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Genome wide association study of psoriasis in India

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Genome wide association studies (GWAS) have identified over 40 psoriasis susceptibility loci in European ancestry populations and 17 in Chinese. Suggestive of genetic heterogeneity, only 9 loci overlap between these two populations. We performed a GWAS of 953 psoriasis cases and 858 controls collected in India on the Illumina OmniExpressExome array. Principal component (PC) analysis showed that the samples are genetically similar to HapMap Gujarati Indian samples. We performed association testing using logistic regression after adjusting for the top 10 PCs. Only the HLA-C region on chromosome 6 showed genome wide significant association in the initial scan. To increase statistical power, we genotyped an additional 849 cases and 614 controls from India on the Illumina CoreExome array, and performed a meta analysis of the two data sets using 735,138 markers that were genotyped in one or both datasets. Two loci achieved genome wide level significance (P<5E-8). The top 9 associated markers are located 30-143 kb telomeric of HLA-C, with the strongest associated marker, rs1265181 (OR=6.2, P=1.9E-103), located 81 kb telomeric. This is in contrast to Caucasian GWAS, where the strongest associated markers map centromeric of HLA-C. The second GW-significant signal was rs6887695 (OR=1.5, P=2.1E-12) near IL12B, an established psoriasis locus. In addition to these two loci, suggestive association signals (P<10E-5) were observed for 18 loci, including markers near RUNX3 and NFKBIA, two loci that have been associated with psoriasis and/or psoriatic arthritis in European-origin populations. These data are currently undergoing additional refinement by imputation and will require verification using an expanded sample set.

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Cutaneous neovascularization in mice with chronic proliferative dermatitis (Sharpin^{cpdm})

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Laboratory mice carrying a null mutation in the *Sharpin* gene develop a systemic hypereosinophilic syndrome with a severe inflammatory and hyperproliferative skin disease called chronic proliferative dermatitis. The inflammatory cells are primarily comprised of eosinophils with lesser numbers of macrophages and neutrophils and an increased number of mast cells. While the mutant mice appear clinically normal at birth, at about 4 weeks of age they begin to develop a rapidly progressive skin disease characterized by hair loss and thick and scaly skin. We evaluated the cutaneous vasculature in these mice from 2 to 10 weeks of age by histology, immunohistochemistry, and transcriptome analyses. While there was no obvious change in the dermal lymphatics (using LYVE1 as a marker), histology and immunohistochemistry using CD31 and smooth muscle actin as markers revealed prominent neovascularization throughout the dermis and the hypodermal adipose tissue. There was progressive upregulation of Vegfa, Flt1, Ccr7, Cxcr4, Pdpn and Sema3a mRNA while the expression of Cxcl12 and Nrp2 mRNA was decreased. Many of the proteins encoded by these genes interact to control angiogenesis and lymphangiogenesis. LYVE1, a marker of lymphatic endothelial cells, remained low consistent with no obvious histologic change. The increased expression of Vegfa mRNA in SHARPIN-deficient mice suggests that VEGFA contributes to the progressive dermal and hypodermal fat angiogenesis observed in these mice.

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Analysis of long non-coding RNAs highlights tissue-specific expression patterns and epigenetic profiles in normal and psoriatic skin

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Although analysis pipelines have been developed to use RNA-seq to identify long non-coding RNAs (lncRNAs), most transcriptome studies of autoimmune disease to date have assessed only protein-coding transcripts due to lack of lncRNA functional annotation. To address this gap, we used RNA-seq data from 99 lesional psoriatic, 27 uninvolved psoriatic, and 90 normal skin biopsies, and applied computational approaches to identify and characterize expressed lncRNAs. We detect 2,942 previously annotated and 1,080 novel lncRNAs which are expected to be skin specific. Notably, over 40% of the novel lncRNAs are differentially expressed and the proportions of differentially expressed transcripts among protein-coding mRNAs and previously-annotated lncRNAs are lower in psoriasis lesions versus uninvolved or normal skin. We find that many lncRNAs, in particular those that are differentially expressed, are co-expressed with genes involved in immune related functions, and that novel lncRNAs are enriched for localization in the epidermal differentiation complex. We also identify distinct tissue-specific expression patterns and epigenetic profiles for novel lncRNAs, some of which are shown to be regulated by cytokine treatment in cultured human keratinocytes. Together, our results implicate many lncRNAs in the immunopathogenesis of psoriasis, and provide a resource for lncRNA studies in other autoimmune diseases.

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MCP-1 is overexpressed by Tsc2-null skin fibroblasts in a mouse model of tuberous sclerosis with targeted disruption of *Tsc2*

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New targets for treating tuberous sclerosis complex (TSC) are needed since patients may not tolerate long-term treatment with mTOR inhibitors. Our previous studies demonstrated that fibroblast-like TSC angiofibroma and periungual fibroma cells produced high levels of MCP-1, a chemokine that promotes tumor growth by stimulating angiogenesis and increasing tumor-associated macrophages. To elucidate the roles of MCP-1 in TSC tumorigenesis and its relationship with loss of TSC2, we utilized a mouse model of TSC skin tumor developed with floxed *Tsc2* alleles and the *cre* recombinase transgene under the control of a *Prrx1* regulatory element (*Prrx1-cre*), which is selectively expressed in craniofacial and limb bud mesenchyme. Fibroblasts isolated from limb and ventral skin of mice with homozygous *Tsc2* floxed alleles and the *Prrx1-cre* transgene (Tsc2cKO mice) lacked Tsc2 protein and had elevated levels of phosphorylated ribosomal protein S6, a marker of mTORC1 signaling. Skin fibroblasts of Tsc2cKO mice released 2.2-fold more MCP-1 into media than fibroblasts from control mice. Skin fibroblasts of Tsc2cKO mice incubated with rapamycin, a specific mTORC1 inhibitor, produced significantly less MCP-1 compared to a DMSO-treated control sample. The skin of Tsc2cKO mice was nearly 2-fold thicker and had more F4/80-positive macrophages than skin of control mice. Levels of MCP-1 in the sera of Tsc2cKO mice were 3-fold higher than in the sera of control mice. These results suggest that MCP-1 may play important roles in TSC tumorigenesis and serve as a novel target for the treatment of TSC.

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Dsp^{rul}: A spontaneous mouse mutation in desmoplakin as a model of Carvajal-Huerta Syndrome

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Studies of spontaneous mutations in mice have provided valuable disease models and important insights into the mechanisms of human disease. A new, autosomal recessive mutant allele of the desmoplakin gene (Dsp) called ruffled (Dsp^{rul}) closely models a rare human disorder, Carvajal-Huerta Syndrome. Carvajal-Huerta Syndrome (CHS) is a rare cardiocutaneous disorder that presents in humans with woolly hair, palmoplantar keratoderma and left ventricular cardiomyopathy. CHS results from an autosomal recessive mutation on the 3′ end of Desmoplakin (DSP) truncating the full length protein. In mice, Dsp^{rul} arose spontaneously in the RB156Bnr/EiJ inbred mouse strain. Mutant mice have an abnormal hair coat, diffuse epidermal blistering, abnormal ECG traces and left ventricular fibrosis as compared to wildtype littermates. High-Throughput Sequencing (HTS) found an insertion at 38,288,978 bp of chromosome 13 predicted to result in a frameshift of Dsp, truncating the last 9.4% of the c-terminal portion of DSP (271 of 2,883 amino acids). The Dsp^{rul} mouse provides a new model to investigate the pathogenesis of CHS, as well as the underlying basic biology of the adhesion molecules coded by the desmosomal genes.

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Meta-analysis of the TNIP1 region in psoriasis identifies two independent association signals

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To further understand the association of tumor necrosis factor alpha-induced protein 3 interacting protein 1 (TNIP1) with psoriasis, we performed a fine-mapping study of the TNIP1 gene region on chromosome 5. TNIP1 encodes the ABIN1 protein which plays a role in downregulating inflammation mediated by the NF-kB signaling pathway. Single nucleotide polymorphisms (SNPs) in the TNIP1 gene region have been shown to be associated with psoriasis and other autoimmune diseases (AIDs) such as systemic lupus erythematosus and systemic sclerosis. Using five psoriasis cohorts of European descent totaling 4,704 cases and 7,805 controls, we imputed a 250kb region surrounding both sides of the TNIP1 gene using a combined reference panel from the Division of Cancer Epidemiology and Genetics (DCEG) and 1000Genomes cohorts. We meta-analyzed the association results of each cohort using a random-effects model. Conditional analyses were performed to find additional signal peaks of association. We then performed a literature search to compare our top SNPs to previously published TNIP1 SNPs associated with other AIDs. The meta-analysis revealed 12 variants that exceeded a Bonferroni-corrected threshold. Our most significantly associated SNP is rs79901336 (odds ratio (OR) = 1.66, p= 1.84e-19). Upon conditioning on that top SNP, we found another independent association at rs17801328 (OR 1.68, p(cond)=7.80e-06). After comparing our top hits with SNPs in other autoimmune diseases, we show that the psoriasis top SNPs are on a different linkage disequilibrium (LD) block than the TNIP1 SNPs associated with other AIDs. We identified an additional novel independent signal peak near TNIP1 associated with psoriasis. The unique variants associated with psoriasis suggest that TNIP1 expression may be regulated by different variants in psoriasis compared to other AIDs.

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Dominant de novo GJA1 mutations cause erythrokeratodermia variabilis

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Genetic investigation of inherited skin disorders has informed understanding of skin self-renewal, differentiation, and barrier function. Disorders of keratinization (DOK), characterized by scaling and frequently associated additional epidermal and systemic sequelae, demonstrate significant phenotypic and genotypic heterogeneity. Over 70 DOK genes have been identified, and yet these genes explain only a portion of observed DOK phenotypes. We have recruited a cohort of over 550 comprehensively phenotyped DOK kindreds, and are employing a tiered approach whereby cases are screened for mutations in known genes, and the remaining cohort is studied utilizing exome sequencing to facilitate novel gene discovery. One example of the success of this effort is the identification of a novel gene causing erythrokeratodermia variabilis (EKV), a rare, inherited skin disease characterized by transient figurate patches of erythema, localized or generalized scaling, and frequent palmoplantar keratoderma. We have found de novo missense mutations in GJA1 (gap junction protein alpha 1) in three unrelated, sporadic EKV subjects, two of which alter the same amino acid. The severe, progressive skin disease in EKV subjects with GJA1 mutations is without overlap with the systemic, non-epidermal disorder oculodentodigital dysplasia, also caused by dominant GJA1 mutations. GJA1 encodes connexin 43 (Cx43), the most widely expressed gap junction protein. GJA1 mutations in EKV subjects lead to disruption of Cx43 membrane localization, and aggregation within the Golgi. These findings reveal a critical role for Cx43 in epidermal homeostasis, and provide evidence of organ-specific pathobiology resulting from different mutations within GJA1.

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Analysis of transcriptomes from palmoplantar pustulosis and palmoplantar pustular psoriasis suggests that they may not be different clinical entities

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There is a controversy surrounding the existence of palmoplantar pustulosis (PPP) and palmoplantar pustular psoriasis (PPPP) as separate clinical entities or as variants of the same clinical entity. We used gene microarray to compare gene expression in patients PPP and PPPP. Skin biopsies from subjects with PPP (3), PPPP (6), psoriasis vulgaris (10) and acral skin from normal subjects (7) were analyzed using gene microarray. Principal component analysis showed that PPP and PPPP were different from psoriasis vulgaris and normal acral skin. However gene expression of PPP and PPPP clustered together. Gene-wise comparison between PPP and PPPP found no gene to be differentially expressed with a false discovery rate lower than 0.6. Surprisingly we found a higher expression of several genes usually expressed in the central or peripheral and nervous system (e.g. GPRIN, ADAM23 and RIMS3) in PPP/PPPP as compared to psoriasis vulgaris and normal acral skin. Immunohistochemistry confirmed these findings and showed that these proteins were mostly located in keratinocytes. In conclusion PPP and PPPP could not be differentiated using gene microarray suggesting that they may not be distinct clinical entities. Increased expression of GPRIN1, ADAM23 and RIMS3 in keratinocytes warrants further evaluation of their potential role in the pathogenesis of PPP/PPPP.

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Psoriasis drug development and GWAS interpretation through in silico analysis of transcription factor binding sites

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Psoriasis is a cytokine-mediated skin disease that can be treated effectively with immunosuppressive agents. These medications, however, are not equally effective in all patients and are poorly suited for treating mild psoriasis. To develop more targeted therapies, interfering with transcription factor (TF) activity is a promising strategy. We have therefore compiled a dictionary of 2935 binding sites representing empirically-determined binding affinities of TFs and unconventional DNA-binding proteins (uDBPs). This dictionary was screened to identify "psoriasis response elements" (PREs) overrepresented in sequences upstream of genes differentially expressed (DEGs) in lesional vs. uninvolved skin of psoriasis patients (n = 237). We show that PRE sequences are recognized by IRF1, ISGF3, NF-kappaB and multiple TFs with helix-turn-helix (homeo) or other all-alpha-helical (high-mobility group) DNA-binding domains. Additionally, we identified a limited set of DEGs that encode proteins interacting with PRE motifs, including TFs (GATA3, EHF, FOXM1) and uDBPs (AVEN, RBM8A, GPAM). PREs were prominent within enhancer regions near cytokine-encoding DEGs (IL17A, IL19 and IL1B), suggesting that PREs might be incorporated into complex decoy oligonucleotides (cdODNs) for therapeutic purposes. To illustrate this idea, we designed a cdODN to concomitantly target psoriasis-activated TFs (i.e., FOXM1, ISGF3, IRF1 and NF-kappaB). Finally, we screened psoriasis-associated SNPs to identify risk alleles that disrupt or engender PRE motifs. This identified possible sites of allele-specific TF/uDBP binding and showed that PREs are disproportionately disrupted by psoriasis risk alleles. These findings identify new TF/uDBP candidates and illustrate an *in silico* strategy that (i) connects transcriptome informatics to cdODN drug development and (ii) enhances our ability to interpret GWAS findings.

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Buschke-Ollendorff syndrome in the absence of LEMD3 mutation

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Buschke-Ollendorff syndrome (BOS) is a rare disorder that classically exhibits connective tissue nevi (collagenomas or elastomas) and osteopoikilosis (OPK). BOS is highly penetrant, but exhibits variable expressivity, resulting in some patients with skin manifestations in the absence of OPK, especially prior to puberty. BOS is autosomal dominantly inherited, resulting from heterozygous loss-of-function mutations in LEMD3, an inner nuclear membrane protein that antagonizes transforming growth factor beta (TGF-β) and bone morphogenetic protein (BMP) signaling. We present an 11-year-old boy with multiple collagenomas without OPK, consistent with BOS. Sanger sequencing of all 13 exons and splice sites within the LEMD3 gene failed to reveal a causative mutation. Copy number variation analysis showed no deletion or duplication involving the LEMD3 locus. Whole exome sequencing did not identify any mutations in LEMD3, nor in the TGF-β and BMP genes or their respective receptors. To our knowledge, this is the second report of a patient with BOS that lacks a mutation in the LEMD3 gene. This case highlights the potential genetic heterogeneity of BOS. Further studies are ongoing to determine other genes that might be responsible for a BOS-like phenotype.

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Functional genomics of the *ULBP6* locus reveals a critical role for CTCF-mediated long-range interactions in alopecia areata

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Alopecia areata (AA) is among the most highly prevalent human autoimmune diseases, characterized by disfiguring hair loss due to the collapse of immune privilege in the hair follicle. To determine the genetic architecture of AA, our lab performed the first Genome-Wide Association Study (GWAS) and identified the *ULBP3/6* locus on chr.6q25.1 ($p = 5.9 \times 10^{-24}$) as the most significantly associated region outside the *HLA*. The *ULBP* genes reside within an MHC class I-related cluster and encode ligands for the NKG2D cytotoxic T cell receptor. We previously reported a striking upregulation of these ligands in both human and mouse AA hair follicles, and more recently, demonstrated that CD8+NKG2D+ cells are both necessary and sufficient to induce disease in the AA mouse model. To identify functional variants at the *ULBP3/6* locus, we performed targeted deep resequencing of this region on 124 AA patients and uncovered a small number of rare, non-coding variants enriched on GWAS risk haplotypes. Interestingly, we identified three novel variants that reside within distal *ULBP6* regulatory elements and localize to CTCF binding sites. CTCF is an insulator involved in chromatin remodeling and gene repression, therefore, we postulate that *ULBP6* non-coding variants disrupt CTCF-mediated long-range interactions required for *ULBP* repression in the hair follicle. *In vivo*, we find that CTCF is highly enriched at *ULBP6* by ChIP-PCR on human scalp dermal fibroblasts, and *in vitro* reporter assays on this candidate region demonstrate strong repressive activity and responsiveness to CTCF binding. Remarkably, these CTCF-*ULBP6* interactions are abrogated in the presence of all three AA-associated *ULBP6* rare variants. Our findings implicate CTCF as a long-range repressor of the *ULBP* locus in the human hair follicle, delineating a potential functional role for these variants in disease susceptibility in AA.

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Multiple facial vellus hair cysts, ear pits, lipomas, macrocephaly, joint laxity and cardiac defects: A novel genodermatosis?

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Eruptive vellus hair cysts (EVHC) often occur on the trunk and limbs. Facial involvement is uncommon. Autosomal dominant inheritance has been described but associated extra-cutaneous anomalies have not. We describe a 4-patient kindred presenting with multiple facial vellus hair cysts, and an association of ear pits, lipomas, macrocephaly, joint laxity and cardiac defects. Patients were interviewed, examined, and photographed with consent. Evaluations performed include skin biopsy (3 of 4 patients), echocardiogram, and electrocardiogram. Cutaneous histopathologic examination confirmed the diagnosis of vellus hair cysts in all 3 affected individuals. Data on clinical findings (lipomas (3 of 4 patients), joint laxity (4 of 4 patients), ear pits (4 of 4 patients), cardiac defects (1 of 4 patients), macrocephaly (1 of 4 patients) were documented. We propose that facial EVHC may indicate the presence of a novel inherited autosomal dominant disorder with multisystemic manifestations.

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Onychodystrophy, Palmoplantar keratoderma, and Amelogenesis imperfecta (OPA) syndrome caused by a homozygous mutation in *CNBD2*

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In the present study, we describe a previously unrecognized autosomal recessive ectodermal dysplasia featuring Onychodystrophy, Palmoplantar keratoderma and Amelogenesis imperfecta (OPA) in a large consanguineous family. Using whole exome-sequencing analysis, we identified a homozygous point mutation (c.1481G>A p.R494Q) in *CNBD2* (cyclic nucleotide binding domain 2) in affected individuals. A number of facts support a pathogenic role for this mutation: (1) the mutation was found to co-segregate with the disease phenotype in the affected family; (2) the mutation is predicted to be damaging; (3) the mutation affects a highly conserved amino acid residue (ConSeq=8, range 1-9); (4) the mutation was not found in public databases (>8,000 individual sequences) and was not detected in a series of 186 population-matched healthy individuals. Confocal microscopy revealed that *CNBD2* is normally expressed in the cytoplasm and nucleus of basal and supra-basal epidermal cells, follicular epithelium and nail matrix. *CNBD2* was found to be up-regulated during epidermal differentiation *in vitro*. Global gene expression and pathway analyses revealed that down-regulation of *CNBD2* expression in primary keratinocytes resulted in disturbed expression of epidermal differentiation genes as well as down-regulation of *TP63* (and many of its target genes), *WNT10a* and *GJA1*, which all encode important mediators of ectodermal differentiation. Taken together, the present results reveal a central role for CNBD2 during ectodermal differentiation.

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The BAF/SWI/SNF complex controls genome accessibility to p63 during epidermal differentiation

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"Open" and "closed" chromatin states impact gene expression by rendering genomic regions either accessible for expression or by repressing them, respectively. Chromatin remodeling complexes, such as the BAF (SWI/SNF) complex, control the transition between these open and closed states. In epidermis, BAF catalytic subunits, Brg1 and Brm, are required for differentiation and are frequently mutationally inactivated in SCC; however, how BAF acts to control differentiation in homeostasis and cancer is unknown. To address this, we mapped chromatin accessibility genome-wide in the presence or absence of functional BAF using ATAC-seq. We found that BAF is required to maintain accessibility of 6.8% of total open chromatin regions during epidermal differentiation. Motif searches of these BAF-dependent regions identified a striking enrichment of the p63 DNA binding motif ($p=1X10^{-209}$). mRNA profiling indicates a significant overlap between the target gene sets of p63 and BAF ($p=2.6x10^{-105}$) with the 424 co-regulated genes enriched for top gene ontology (GO) terms including "epidermal development" ($p=3.9x10^{-9}$) and "epidermal cell differentiation" ($p=1x10^{-5}$). Our ATAC-seq studies demonstrated that BAF loss led to loss of accessibility, at 40 basepair average compaction, selectively at p63 binding sites. Binding sites of other factors, such as CTCF, was unaffected, underscoring that BAF effects on genome accessibility are selective for p63. BAF loss decreased p63 genome binding at its target sites and vice versa, demonstrating that genomic binding by p63 and the BAF complex is cooperative. Genome-wide ChIP-seq analysis of active and repressive marks indicates that BAF loss also decreases marks of active chromatin, such as H3K27Ac, p300, and RNA Pol II, at p63 binding sites, while increasing the repressive H3K27me3 mark at these sites. Therefore our data demonstrates that the BAF complex and p63 cooperatively bind to maintain open chromatin and promote epidermal differentiation.

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Novel regulatory variants identified in adult atopic dermatitis by targeted deep sequencing alter enhancer function

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Atopic Dermatitis (AD) is a common heritable, inflammatory skin disease. Although filaggrin (*FLG*) mutations are a strong risk factor for AD, susceptibility loci (1q and 11q) underscore additional genetic factors. Motivated by recent studies identifying enrichment for noncoding regulatory variation in common disease, we hypothesized a role for 1q and 11q regulatory variants in AD using targeted next-generation sequencing (NGS). We prioritized variant discovery in adult patients of a specific AD endotype - baseline skin barrier impairment (downregulation of Epidermal Differentiation Complex [EDC] genes) and immune abnormalities. Long-range PCR genotyping revealed fewer *FLG* intragenic monomer repeats in AD patients vs. controls ($p=0.018$) and few *FLG* mutations. Targeted NGS of 5 ENCODE-annotated enhancers and 4 functional regulatory elements (REs) in linkage disequilibrium with GWAS SNPs (rs877776, 1q21; rs7927894, 11q13.5) identified 17 regulatory variants (15 common SNPs, 2 novel EDC variants) in our pooled AD cases. Multiplex Sequenom genotyping assigned the novel EDC regulatory variants (in RE 180 and 184) to 2 independent patients with severe AD (SCORAD $\geq$50). Family pedigree analysis for proband 2 demonstrated segregation of the novel RE 184 variant with disease but with incomplete penetrance. Pairwise comparisons between minor and major regulatory alleles in cell-based reporter assays revealed greater effect sizes for the novel compared to the common minor variants. Moreover, chromatin conformation capture studies identified specific chromatin interactions with target EDC genes for RE 180 and 184. Many of the identified target genes are differentially expressed in the two patients that each harbor the respective novel variant. To our knowledge, our study is the first to report a functional role for novel regulatory variants in a distinct molecular endotype for AD and increases our understanding of the genetic architecture of AD.

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Guanine nucleotide binding protein alpha q polypeptide ($Gnaq^{M1J}$): An ENU induced mutant allele affecting dermal melanocytosis in the mouse

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In an N-ethyl-N nitrosourea (ENU) mutagenesis screen at The Jackson Laboratory, a colony of C57BL/6J mice was examined with unusually darkly pigmented skin on the body, tail, ears, lower legs, and foot pads. Histological examination revealed increased interfollicular epidermal pigmentation and dermal pigmentary incontinence not seen in wild type littermates. Sequencing identified an A to G missense mutation in exon 2 in the Gnaq (Guanine Nucleotide Binding Protein (G Protein), Q Polypeptide) gene. Previously reported ENU induced mutations in Gnaq ($Gnaq^{Mhdadsk1}$ and $Gnaq^{Mhdadsk10}$) also showed increased pigmentation of the skin, but hyperpigmentation was limited to the dermis, unlike the $Gnaq^{M1J}$ allele. Somatic mutations in Gnaq were recently linked to Sturge-Weber Syndrome, port wine stains, blue melanocytic nevi, and dermal melanocytosis (Mongolian spots). This new allelic mutation provides yet another tool to study the development and maturation of melanocytes in the skin.

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Skin fragility of the wild-derived, inbred mouse strain *Mus pahari*

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Mus pahari is a wild-derived, inbred strain of mouse imported to The Jackson Laboratory from the National Cancer Institute (NCI) in 1995. It is primarily used in the field of virology because of a functional xenotropic and polytropic retrovirus receptor 1 (*Xpr1*) gene which makes this strain susceptible to xenotropic murine leukemia virus-related virus (XMRV). For many years, *M. pahari* colony managers have known about the fragility of this strain's skin resulting in separation of tail skin from the mouse if handled incorrectly. Tail skin tension testing of *M. pahari* resulted in significantly lowered force threshold in comparison to closely related inbred mouse species and subspecies. Histologically the tail skin separated at the subdermal level with the dermis firmly attached to the epidermis, excluding the epidermolysis bullosa complex of diseases. The dermal collagen bundles were abnormally thickened and branched. Elastin fiber deposition was focally altered in the dermis adjacent to the hair follicle. Collagens present in the skin could not be differentiated between the species in protein gels. Together these data suggest that *M. pahari* have altered extracellular matrix development resulting in separation of the skin below the level of the dermis with moderate force similar to the African spiny mouse (*Acomys*).

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Mouse models of skin and adnexal diseases in the Mouse Mutant Resource (MMR) at The Jackson Laboratory

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The Mouse Mutant Resource (MMR) at The Jackson Laboratory (JAX) has been in existence for over 50 years with continuous support by the National Center for Research Resources (NCRR) since 1978. Mice carrying spontaneous mutations have been a rich resource for productive skin and adnexal disease research since the early 1900s. Despite the capability in recent decades to genetically engineer mouse models, strains harboring spontaneous mutations affecting the skin and adnexa continue to provide the raw material for in-depth investigator-initiated research, broad phenotypic insight, nucleotide-level functional annotation of the mouse genome and faithful modeling of common and rare human genetic disorders. The hundreds of thousands of mice on the JAX campus on any given day, and the millions of mice produced over the course of a year, offer a unique and powerful opportunity for the dermatology research community to access spontaneous mutation mouse models. Within such large populations, spontaneous mutations inevitably occur, and at a detectable rate not fully appreciated in smaller colonies. These spontaneous mutations are manifested in mice (known as phenotypic deviants) with rare, unusual and biomedically relevant skin phenotypes. The JAX MMR has an established and productive program for discovering, characterizing and distributing these models to dermatology researchers worlwide.

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Inherited LCK deficiency causes susceptibility to EV-HPV infections and early-onset squamous cell carcinoma

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ABSTRACT Inherit edepidermodysplasia verruciformis (EV) is a rare skin disorder characterized by susceptibility to specific types of human papilloma virus (HPVs) and is strongly associated with non-melanoma skin cancer. Inactivating mutations in EVER1 or EVER2 genes have been detected in most, but not all EV patients. In this study, we found 3 young EV siblings presenting with persistent EV-HPV infections, T cell defects and early-onset squamous cell carcinoma (SCC) without EVER1/2 mutation. These specific clinical manifestations suggest that they displayed significant heterogeneity compared with common EV patients. Thus we applied whole exome sequencing (WES) to identify potential mutation(s) account for EV-HPV infections in this family. A novel homozygous splicing mutation has been detected in the T lymphocyte-specific protein tyrosine kinase (LCK) gene (c.188-2A>G), which resulted in expression of only mutant but not wild-type LCK in all patients. Our results demonstrate mutations in LCK are associated with EV and provide new clues for the understanding of host defenses against HPV and better genetic counseling of EV patients.

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Intra-familial variation in clinical phenotype of *CARD14*-related psoriasis

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Psoriasis is a chronic multifactorial inflammatory disease involving skin, nails and joints. Recently monogenic forms of the disease have been described, including dominantly inherited plaque type and generalized pustular psoriasis, related to activating mutations in the CARD14 gene. In the present study we describe a family with multiple cases of dominantly inherited psoriasis, an extreme variability of clinical presentation (from mild plaque type to generalized pustular psoriasis) and early disease onset. The aim of the study was to investigate the genetic basis of psoriasis in the affected family members and to propose possible mechanisms for the intra-familial phenotype variability. Screening of the affected individuals for mutations in the coding regions and the exon-intron boundaries of the IL36RN, IL1RN and CARD14 genes revealed the c.349G>A [p.Gly117Ser] mutation in the CARD14 gene, previously reported to be associated with plaque type psoriasis. The most severely affected family members had three additional coding region polymorphisms (rs2066964 [c.1641G>C; p.Arg547Ser], rs34367357 [c.1753G>A; p.Val585Ile] and rs11652075 [c.2458C>T; p.Arg820Trp]) in the CARD14 gene, suggesting their possible effect on the disease expression. In the aforementioned family early onset psoriasis co-segregated with the HLA-C*0602, indicating that the presence of the HLA-C*0602 could potentially modulate the time of the disease onset. In summary: In the current work we describe a family with *CARD14*-related psoriasis and discuss the possible influence of the specific haplotype of the affected family members on the intrafamilial variation in the clinical phenotype of the disease.

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Mutations affecting keratin 10 surface exposed residues highlight the structural basis of phenotypic variation in epidermolytic ichthyosis

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Epidermolytic ichthyosis (EI) due to *keratin-10* (*KRT10*) mutations is a rare, typically autosomal dominant, disorder characterized by erythema and blistering at birth followed by hyperkeratosis and less frequent blistering later in life. We identified two previously unreported *KRT10* mutations p.Q434del and p.R441P in subjects presenting with a mild EI phenotype without skin fragility at birth. Both mutations occur within the mutational "hot spot" of the keratin 10 2B rod domain, adjacent to mutations which cause more severe phenotypes. p.Q434del and p.R441P form collapsed K10 fibers rather than the aggregates observed in severe EI. Upon calcium-induced differentiation primary keratinocytes isolated from the p.Q434del case showed significantly lower apoptosis (p-value <0.01) compared to p.R156C primary keratinocytes as assessed by TUNEL assay. Conversely, the mitotic index of the p.Q434del epidermis was significantly higher compared to the severe EI epidermis (p-value <0.01) estimated by Ki67 staining. We sought to interrogate the pathophysiology of these mutations using structural modeling of interaction between K10 and its binding partner keratin-1 (K1), but finding no reported crystal structure, we employed homology-based modeling to generate wild type (WT) and mutant 2B domain models. Such models suggested that the mutations in our p.Q434del and p.R441P subjects affect surface exposed residues of the alpha helix coiled-coil and cause localized disorganization of the K1-K10 heterodimer. In contrast, models of severe EI mutations including p.R156C suggest disruption of key inter-molecular interactions between K1 and K10. This study provides insight into the structural basis of the phenotypic variation in EI due to *KRT10* mutations.

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Systems biological analysis of alopecia areata reveals master regulators of hair follicle immune privilege

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Alopecia areata (AA) is an autoimmune disease characterized by hair loss due to cytotoxic T-cell-mediated destruction of hair follicles. While aberrant immune activity clearly contributes to the disease, clinically, AA patients are generally otherwise healthy, suggesting that local genetic elements in the skin may mediate end-organ-specific infiltration recruitment. This end organ hypothesis predicts that the interaction between cytotoxic-T cells and the hair follicle may be mediated at least in part by molecular changes in the skin that cause the loss of its immune privilege from the host's immune system. Gene expression analysis of an AA cohort revealed distinct molecular programs implicating a wide array of biological processes including, but not limited to, canonical immune-response signaling pathways. With hypothesis that some portion of this molecular program is required for immune infiltration, we sought to model the regulatory interactions leading to those molecular behaviors. We generated a regulatory model for the molecular behavior of skin and hair follicles in the context of AA using the ARACNe algorithm designed in cancer research and a microarray data of the skin and hair. Using these models, we have isolated specific molecular programs that define AA initiation and searched for the minimum number of genetic regulators required to reproduce these gene signatures. We have identified the transcriptional regulators IKZF1 and DLX4 as master regulators sufficient to induce an AA-like gene expression signature in normal cell types. These regulators are found aberrantly expressed in patients AA and exhibit suggestive association to AA in GWAS studies. Their expression in normal cells enhanced NKG2D-dependent cytotoxic killing in two independent cellular contexts. Using computational models, we have recreated an end-organ molecular regulatory program that appears sufficient to mediate and recruit an AA-like, T-cell-dependent immune response.

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A role for autocrine and paracrine action of the Th1 chemokines in the pathogenesis of keratoderma

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The underlying causes of keratoderma, a painful, disfiguring and progressive disease, are not well understood. Our recent studies indicate that inactivation of AP1 transcription factor activity in the suprabasal epidermis produces a murine phenotype that mimics human keratoderma. The phenotype is characterized by hyperproliferation, hyperkeratosis, parakeratosis, pseudoainhum of the tail and digits, and nuclear localization of loricrin. Nuclear loricrin localization and pseudoainhum are signature phenotypes of some types of keratoderma. We hypothesized that altered epidermal chemokine production may, in part, be responsible for the disease phenotype. Array analysis reveals a selective substantial increase in production of the interferon-gamma inducible Th1 chemokines, CXCL9, CXCL10 and CXCL11. We proposed that these chemokines interact with the CXCR3 receptor to stimulate lymphocyte recruitment and keratinocyte hyperproliferation. Consistent with this idea, we observe enhanced epidermal T cell recruitment to the epidermis, and enhanced proliferation of CXCR3-positive basal keratinocytes. These findings suggest that Th1 chemokines may stimulate keratinocyte proliferation and promote Th1 lymphocyte accumulation to contribute to the keratoderma phenotype.

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Pathway analysis and protein-protein interaction network construction provide functional interpretation of GWAS evidence in alopecia areata

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GWAS have successfully identified 14 genomic loci with association to alopecia areata (AA) that range in size from 30Kb to 500Kb, containing multiple genes and regulatory features that can influence genes outside of the associated LD block. The major challenge in translating this evidence is determining which gene/s at or near a GWAS locus are causal. Pathway and network analyses have been used to prioritize genes at GWAS loci and provide insight into disease pathogenesis, implicating biological processes of etiologic importance. Here, we performed pathway analysis and constructed protein-protein interaction (PPI) networks for the 226 protein coding transcripts located within +/- 500 Kb of the most significant SNP for each GWAS locus. Enriched pathways include Antigen Presentation (n=16; $p=3x10^{-12}$), JAK-STAT Signaling (n=10; $p=9x10^{-4}$), and Costimulatory Signaling (n=6; $p=1x10^{-5}$). Enriched gene ontology (GO) terms include MHC class II (n=12, $p=1x10^{-14}$) and class I (n=6, $p=9x10^{-7}$) proteins, T-cell activation (n=10, $p=1x10^{-5}$) and differentiation (n=6, $p=4x10^{-4}$), and regulators of transcription (n=12,p=.03) and post translational modifications (n=13, p=.02). Also, some cellular processes that did not reach significance are implicated by genes across loci and include redox (10 genes, 5 loci), apoptosis (10 genes, 7 loci), and steroid hormone signaling (7 genes, 6 loci). Construction of a PPI network identified 40 direct connections (p=.007) among 46 genes from 13 of the 14 GWAS loci. Allowing for a common interactor generated a single highly connected network (p=.002) of 148 genes from all 14 GWAS loci. The results of pathway analysis and PPI network construction allow us to contextualize statistical evidence from GWAS within a biological framework and to prioritize candidate genes for further genetic studies (e.g. targeted resequencing). Importantly, these results also support our ongoing clinical research which shows human clinical relevance for therapeutics targeted to the Costimulatory (e.g. Abatacept) and JAK/STAT (e.g. Ruxolitinib, Tofacitinib) pathways.

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Juxta-articular joint-capsule mineralization in CD73 deficient mice: Similarities to patients with *NT5E* mutations

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Arterial calcification due to CD73 deficiency (ACDC), an autosomal recessive disorder, manifests with extensive mineralization of the lower-extremity arteries as well as of hand and foot joint-capsules. This disease is caused by mutations in the *NT5E* gene which encodes CD73, a membrane-bound ecto-5′-nucleotidase hydrolyzing 5′-AMP into adenosine and P_i. To gain insight into the pathophysiologic details of ACDC, we have characterized a *Nt5e*$^{-/-}$ knock out mouse (*Nt5e*^{tm1Jgsc}) deficient in CD73. These mice, when maintained on appropriate strain background, demonstrated stiffening of the joints and micro computed tomography revealed distinct changes in the thoracic skeletal structure with evidence of mineralization at the costochondral junctions. Mineralization was also noted in the juxta-articular spaces of the lower extremities as well as of ligaments and capsules adjacent to the bony structures. No evidence of vascular mineralization was noted either by micro-computed tomography or by microdissection of arteries in the thoracic area or in lower extremities. The *Nt5e*$^{-/-}$ mutant mice demonstrated significantly increased P_i levels in the serum and significantly reduced PP_i concentration in the heparinized plasma, resulting in markedly increased P_i/PP_i ratio, thus creating a pro-mineralization environment. In conclusion, the *Nt5e*$^{-/-}$ targeted mutant mice recapitulate some, but not all, features of ACDC and serve as a model system to study pharmacologic interventions for ectopic vascular mineralization. Collectively, this mouse model deficient in CD73, with other targeted mutant mice with vascular mineralization, attests to the presence of a complex pro-mineralization/anti-mineralization network that under physiologic homeostatic conditions prevents ectopic tissue mineralization.

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miR29b1 plays an important role in epidermal cell growth and survival

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MicroRNAs (miRNAs) are a group of small noncoding RNAs that interact with specific mRNAs to regulate gene expression. A number of miRNAs have been characterized as key regulators of cell growth and survival. It is unclear how miRNAs are regulated by AP-1 gene regulators during epidermal growth and differentiation. In this study, we performed ChIP-seq analysis with human keratinocytes and identified 12 miRNAs whose gene promoter regions showed >50 fold enrichment by ChIP with a c-Jun antibody. Among these, miR29b1 was highly expressed in proliferating keratinocytes. By RT-PCR, we found that miR29b1 level was markedly decreased upon c-Jun gene silencing and increased by c-Jun overexpression. Also in line with the reduced level of active c-Jun (namely pc-Jun), miR29b1 was decreased in response to Ca^{++}-induced epidermal differentiation. Functionally, overexpression of miR29b1 rescued cells from apoptosis induced by c-Jun loss, which was correlated with reduced expression of Bim1, a proapoptotic molecule previously identified as a miR29-target in neuronal cells. Moreover, c-Jun gene silencing increased, while overexpression of miR29b1 decreased expression of CAMK2G, an important regulator of Ca^{++} signaling. Lastly, miR29b1 was expressed at an elevated level in A431 SCC cells compared to keratinocytes, and its exogenous expression prevented A431 cell death and growth arrest induced by c-Jun loss. Taken together, these findings indicate that miR29b1 is positively regulated by c-Jun, and plays important roles in cell growth and survival of both normal keratinocytes and cancer cells. Currently, our efforts are focused on confirming the functional importance of miR29b1 in epidermal growth and differentiation by using 3-D culture and skin grafting.

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Kindler syndrome: Novel and recurrent *FERMT1* mutations in 20 unique families with 70 patients and evidence of genetic heterogeneity

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The Kindler syndrome (KS) is a rare autosomal recessive genodermatosis characterized by diffuse poikiloderma, cutaneous atrophy, acrokeratosis, trauma-induced blisters and photosensitivity. Sixty two mutations have been previously disclosed in the *FERMT1* gene in ~150 patients; this gene encodes kindlin-1, a component of keratinocyte focal adhesions. The incidence of KS is expected to be relatively high in Iran due high rate of consanguineous marriages. We examined a total of 20 Iranian families with KS, including 70 affected individuals, including one extended family with 24 patients. Sequencing of *FERMT1* by amplification of all 15 exons and flanking intronic sequences as well as the promoter region, revealed 14 distinct mutations in 17 families, 11 of the mutations being novel. Two novel mutations, p.Q226X and c.1253delA, were found in more than 1 family (4 and 2, respectively). Haplotype analysis of the *FERMT1* region in these families indicated the presence of a conserved haplotype, indicating founder effect. In two families, no mutation in *FERMT1* gene was found and autozygosity mapping excluded this locus. Careful examination of patients in these two families revealed unusual clinical features suggesting new entities with clinical overlapping features with KS. Morpholino knockdown of *Fermt1* in zebrafish results in a ruptured fin phenotype, forming the basis to test the pathogenicity of 3 thus far identified missense mutations in *FERMT1*. Collectively, the previously unpublished mutations in the *FERMT1* gene expand the spectrum of mutations underlying KS. The identified mutation database forms the basis to confirm the clinical diagnosis by genetic testing and to identify heterozygous carriers, which when coupled with genetic counseling can reduce the burden of KS in Iran.

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Somatic V600E *BRAF* mutation causes syringocystadenoma papilliferum

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Syringocystadenoma papilliferum (SCAP) is a benign skin tumor with rare progression to malignancy and has histology characterized by cystic epithelial hyperplasia, papillary projections lined by columnar epithelium, and a plasma cell infiltration in surrounding stroma. SCAP can arise spontaneously as a solitary neoplasm, or may be present at birth in a linear configuration, following the dorsovental patterns of migration of keratinocyte precursors, known as lines of Blaschko. Expecting that such linear lesions result from post-zygotic mutation during early development, we performed whole exome sequencing of a Blaschkoid SCAP, finding a single damaging tissue-specific mutation, *BRAF* V600E. Targeted sequencing of 9 spontaneously arising SCAPs, revealed the identical BRAF V600E mutation in 4 additional samples. These data demonstrate that both mosaic and sporadic SCAP can be caused by somatic BRAF mutation and raise the possibility that rare malignant examples of SCAP lesions might respond to molecular therapeutics targeting the BRAF V600E mutation.

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The spectrum of *COL7A1* mutations identified in 63 families with dystrophic epidermolysis bullosa by comparative Sanger and next generation sequencing

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Epidermolysis bullosa (EB) is a clinically and genetically heterogeneous group of heritable blistering diseases caused by mutations in at least 18 genes. There are 4 types of EB based on the ultrastructural level of tissue separation: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and the Kindler syndrome. There is currently no comprehensive study about the molecular epidemiology of EB in Iranian populations. Clinical examination and immunofluorescence mapping showed that EBS, JEB, and DEB represent 13, 15, and 66% of the Iranian cohort, respectively. Due to high rate of consanguineous marriages these ratios are very different from EB patients in Europe and the US in which EBS, JEB, and DEB were reported to be 40, 27, and 33%, respectively. In the present study, we are assessing a cohort of 140 DEB families for pathogenic sequence alterations in the *COL7A1* gene. Results from Sanger sequencing of 63 families show 46 distinct mutations, 23 being previously unreported. Among the mutations 14 were missense, 14 small indels, 4 splice-junction mutations, 11 nonsense, and 3 large indel mutations. Targeted sequencing of *COL7A1* was also performed on the IonTorrent PGM, using an AmpliSeq 3-pool primer design designed to capture all *COL7A1* exons plus 50-bp flanking regions; this approach verified the mutations discovered in a subset of patients by Sanger sequencing. Thirteen mutations were recurrent and found in more than 1 family; a recurrent c.6269_6270delC (p.Pro2090LeufsX116) was found in 8 families. Haplotype analysis of the *COL7A1* locus on chromosomal region 3p21.1 identified conserved haplotypes indicating a founder effect. Our data emphasize the need for population-specific diagnostic and management approaches for EB.

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Evidence for coordinate regulation of Hmga2 and Tlr4 in hair follicle stem cells

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Toll-like receptor 4 (Tlr4) has emerged as a stem cell gene of interest in the mouse Ksc2 locus. The receptor is widely recognized as an essential element in the triggering of innate immunity, binding pathogen-associated molecules such as lipopolysaccharide (LPS), and initiating a cascade of pro-inflammatory events. However the functional role of its differential expression in hair follicle stem cells remains a mystery. To address the problem, we compared differential Tlr4 expression in keratinocytes (KCs) among Tlr4 wild type (C3H/HeOuJ), Tlr4 mutation (C3H/HeJ ; has mRNA but no protein) and Tlr4 deletion (C57Bl/10ScNJ ; neither mRNA nor protein) strains of mice. We found similar coordinate expression between Hmga2 and Tlr4 mRNA in keratinocytes between Tlr4 wild type and Tlr4 mutation strains (p=0.7241 for Hmga2, 0.5219 for Tlr4). As expected, no Tlr4 mRNA expression and low Hmga2 mRNA expression was found in unsorted Tlr4 deletion (p=0.0245 for Hmga2 between Tlr4 wild type and Tlr4 deletion, 0.0244 between Tlr4 mutation and Tlr4 deletion). After sorting with CD34 and CD49f antibodies in Tlr4 wild type and Tlr4 mutation mice, Hmga2 expression in CD34+CD49f+ hair follicle stem cells is more than 30 times higher than that in CD34-CD49f+ base cells (p=0.00016 for Hmga2 between CD34-CD49f+ and CD34+CD49f+). Tlr4 wild type and Tlr4 mutation CD34+CD49f+ and CD34-CD49f+ KCs have significantly higher Hmga2 expression than in Tlr4 deletion KCs (p=0.0409 for Hmga2 between Tlr4-wild type and Tlr4 mutation and Tlr4 deletion KCs, 0.02162 for CD34-CD49f+ KCs and 0.03639 for CD34+CD49f+). In addition, the results were confirmed with macrophages from the three stains (p=0.005 for Hmga2 between Tlr4-non deletion and Tlr4 deletion macrophage, 0.8526 for F4/80), but no Hmga2 protein was observed in macrophages. These results suggest that Hmga2 mRNA is coordinately regulated by Tlr4 mRNA in mouse KCs and macrophage, but the mechanism must be elucidated further.

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Mineralization of soft connective tissues and cartilage in Enpp1*asj-2J* mutant mice

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Generalized arterial calcification of infancy (GACI), an autosomal recessive disorder caused by mutations in the *ENPP1* gene, is characterized by extensive mineralization of the cardiovascular system. The affected individuals in most cases die within the first year of life, and there is currently no effective treatment for this disorder. In this study, we characterized a spontaneous mutant mouse, *asj-2J*, as a model for GACI. These mice were identified as part of a phenotypic deviant search in a large-scale production colony of BALB/cJ mice at The Jackson Laboratory. They demonstrated a characteristic gait due to stiffening of the joints, with phenotypic similarity to a previously characterized *asj* (''ages with stiffened joints'') mouse, caused by a missense mutation in the *Enpp1* gene. Complementation testing indicated that *asj-2J* and *asj* were allelic. PCR-based mutation detection strategy revealed in *asj-2J* mice a large, 40,035 bp, deletion spanning from intron 1 to the 3'-untranslated region of the *Enpp1* gene, coupled with a 74 bp insertion. This was accompanied with a significant reduction in the plasma pyrophosphate concentration and pyrophosphate to phosphate ratio. As a consequence, extensive ectopic mineralization affecting the arterial vasculature, a number of internal organs, and the dermal sheath of vibrissae, a progressive biomarker of the ectopic mineralization process, was demonstrated by a combination of micro-CT, histopathology with calcium-specific stains, and direct chemical assay of calcium. Besides soft connective tissues, micro-CT and histological analysis of these mice also revealed mineralization of cartilage in the ear pinna and trachea. Collectively, the *asj-2J* mouse, characterized by extensive tissue mineralization, serves as a novel model for GACI, a currently intractable disorder.

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MicroRNAs involved in the pathogenesis of pachyonychia congenita

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Pachyonychia congenita (PC) is a dominant negative skin disorder caused by mutations in inducible keratin genes, including *KRT6A*, *KRT6B*, *KRT16* and *KRT17*. The major symptoms are thickened dystrophic nails, leukokeratosis, and painful plantar keratoderma. To better understand PC pathogenesis at the molecular level, RNA profiling was performed on biopsies taken from both involved and uninvolved plantar skin of PC patients. 44 microRNAs were identified to be differentially-expressed (≥ 2.5-fold change) in PC-involved skin. We selected 7 of these microRNAs (miR-203, miR-135b, miR-1246, miR-31, miR-143, miR-199a, miR-199b) and analyzed their mimics and anti-miR inhibitors to further investigate their effects on epidermal morphology. Keratinocyte growth and scratch assays were performed to examine their role in proliferation and migration in an *in vitro* monolayer keratinocyte culture. Induced proliferation could be observed for miR-31, miR-199a, miR-199b and miR-1246 mimics in epidermal keratinocytes. Reduced proliferation was observed for miR-135b. Scratch assay analysis revealed that miR-203, miR-135b, miR-1246, miR-199a and miR-199b mimics reduced the migration rate, whereas miR-143 lead to increased mobility. Experiments are underway to analyze the effect of these microRNAs on skin morphology using 3D epidermal skin equivalents. These results suggest that the identified microRNAs have an influence on proliferation, migration and likely epidermal structure and might be new targets for the treatment of pachyonychia congenita.

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Identification of MITF regulated microRNAs in melanoma

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Microphthalmia associated transcription factor (MITF) is the master transcriptional regulator and regulates several key differentiation and survival functions of melanocytes. MITF is also known to be a lineage addiction oncogene in a subset of melanomas and play a role in drug resistance. However, the precise role MITF plays in melanoma is yet to be elucidated. In order to understand the role of MITF in melanoma, we set out to identify MITF-regulated microRNAs. We hypothesized that a subset of miRNAs are differentially regulated by MITF in melanocytes and melanoma. To identify MITF regulated miRNAs, we first defined putative MITF promoter regions using previously published ChIP-seq binding data and filtered the set with experimentally verified gene expression using siRNA knockdown and RNA-seq. We then applied a filter for evolutionary conservation for M-box and identified 52 (out of 1871 known) potential MITF-regulated miRNAs. To validate regulation by MITF, we used ChIP-seq data to identify MITF binding within the putative promoter regions of all miRNAs and identified 62 miRNAs with MITF binding within the putative promoter regions. We found a statistically significant number of shared miRNAs between computational and ChIP-seq methods, suggesting that microRNAs identified by motif search and refined using evolutionary conservation data potentially form a group of critical MITF regulated miRNAs. Within and around many of the ChIP-seq identified MITF-binding regions in the DNA, we found the presence of Factorbook binding motifs for ~70 different transcription factors, including YY1, E2F1 and CTCF. Expression histograms of miRNAs containing M-boxes plotted using data from TCGA datasets showed positive skew due to a fraction of tumors showing greatly increased expression as compared to the rest, suggesting that a subset of tumors have regulatory mechanisms that cause increased expression of specific miRNAs. Experimental validation of subsets of these putative MITF-regulated miRNAs and their target mRNAs will provide important insight into the role of MITF in melanoma.